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Relationship between Azinphos-Methyl Usage and Residues on Grapes and in Wine in Australia

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Abstract: Azinphos-methyl was applied to Shiraz winegrapes by commercial high-volume and hand-held sprayers during seasons 1993/94 and 1994/95. Residue levels in grapes resulting from treatments applied by commercial sprayer were below the maximum residue level (MRL) of 2 mg kg^{-1} for grapes in Australia, whereas residues resulting from treatments applied by hand-held sprayer still exceeded the MRL five weeks after final application. There was a strong correlation for most treatments between treatment concentration of azinphos-methyl and residue level in grapes, and in wine made from treated grapes. Applied at the recommended rate (1·2 g litre^{-1} wettable powder (WP) and 2·4 ml litre^{-1} suspension concentrate (SC)) by commercial high-volume sprayer, azinphos-methyl residue levels in wine were well below the MRL, and below the MRLs of most importing countries, except Denmark and Sweden. When applied by hand-held sprayer, residue levels in wine were 5·9–29·6 fold higher than those previously obtained by commercial application of insecticide. Since wines are often blends from different grape blocks and grape-growing districts, in practice, this is unlikely to be of concern. Wine made from grapes treated by commercial sprayer showed no detectable residues of azinphos-methyl after one year of storage. In both years, residue levels in grapes of both formulations of azinphos-methyl fluctuated during the five-week post-treatment period, although there was an overall downward trend. Previously unrecorded systemicity in azinphos-methyl was demonstrated in laboratory studies with barley seedlings, and this may explain these fluctuating data in grapes. The reduction of azinphos-methyl residues in grapes over time appears to be a complex phenomenon involving translocation of active ingredient combined with an increase in the size and weight of berries, producing fluctuating residue levels. © 1998 SCI

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Key words: azinphos methyl; residues; grapes; wine

1 INTRODUCTION

Azinphos-methyl insecticide was first registered for use in 1956, in the US on cotton, and has since found widespread use in agriculture on fruit, field and forage crops.[1] The insecticidal action of the chemical is by contact and ingestion.[2] Overseas, azinphos-methyl has been used for the control of berry moth, flea beetle, grape cane girdler, leafhoppers, mealybugs, mites and thrips in grapes. In Australia, fig longicorn *Acalolepta vastator* (Newman) (Coleoptera: Cerambycidae) is a major pest of wine grapes in the Hunter Valley, New South Wales (NSW).[3,4] A control management strategy for this pest using azinphos-methyl at the rate of 1·2 g litre^{-1} wettable powder (WP) or 2·4 ml litre^{-1} suspension concentrate (SC) against newly hatched larvae infesting the trunk and arms has been developed (Goodwin, unpublished data). In Australia, azinphos-methyl has a maximum residue limit (MRL) of

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2 mg kg^{-1} on grapes, but no limits are stipulated for wine.

Azinphos-methyl is regarded as being reasonably stable, with the relative withholding period from the time of last spray to harvest ranging from a few hours to 35 days, depending on the crop and number of spray applications. It does not convert to the oxygen analogue on plant surfaces but can readily degrade to hydrolytic products in the presence of light and moisture.[1] The half-life of azinphos-methyl ranges from three days on beans to 400 days on oranges, where it is absorbed into the surface waxes.[1] In water, residue persistence is pH-dependent and residues are undetectable after 14 days at pH 8.[1,5]

This detailed study was undertaken to determine the relationship between azinphos-methyl usage and the residues on grapes and in wine.

2 EXPERIMENTAL METHODS

2.1 Field treatment

A level block of 24-year-old Shiraz grapes at Pokolbin, Hunter Valley, NSW, was selected for trials in 1993/94 and 1994/95. Whole rows of wine grapes (50–150 vines) were selected as treatment plots, to provide sufficient fruit for five grape samples, plus a further sample to produce wine for analysis of azinphos-methyl residues.

Treatments comprising azinphos-methyl 350 g kg^{-1} WP (Bayer Australia) formulation at 0·7, 1·2 and 2·4 g litre^{-1} and azinphos-methyl 200 g litre^{-1} SC (Bayer Australia) at 1·2, 2·4 and 4·8 ml litre^{-1} were randomly assigned to plots within each of three replicates. In 1993/94 treatments were applied twice, one week apart, to both sides of the row, by an over-row, recycling, high volume sprayer with hollow-cone nozzles operated at 120 psi and delivering 300 litres ha^{-1}. In 1994/95 treatments were hand-applied using a double-nozzled jet with a Sylvan 75-litre Ute-pack battery-powered electric sprayer to test the effect of a more targeted method of application on residue levels in grapes.

A representative sample of grapes (2 kg) was removed for analysis as the control sample, prior to the first treatment being applied. For azinphos-methyl analysis, approximately 1 kg of grapes representative of each treatment plot was sampled from the distal part of several bunches on five weekly occasions, commencing immediately after the final application. The grapes were chilled and transported to the laboratory within one hour of harvest, where they were frozen and stored (−18°C), prior to residue analysis.

2.2 Wine making

A separate sample comprising 1 kg of grapes from each treatment plot was taken two weeks after the second spray in 1993/94, and three weeks after the second spray in 1994/95. The samples were transported to the Horticultural Research and Advisory Station, Gosford, NSW, where the three replicates of each treatment were bulked into 3 kg of fruit. Grapes were then separated from the bunch stalk, crushed and processed. The mixture of juice and skins from each treatment was inoculated with yeast culture (150 ml) to initiate fermentation, and held in a sealed bucket (10 litre) with an air-lock in each lid, for fermentation to occur. The mixture was cap-punched daily for five days, after which the juice was separated from the skins and seeds using a wine press, yielding 2 litres of wine per treatment. The wine was then placed in a 2-litre sealed conical flask with an air-lock in the bung, and the sugar content monitored daily using an Atago RX3P digital refractometer. Percentage of reducing sugar in the wine was determined using ‘Clintest’ tablets to indicate cessation of fermentation. The wine was then placed in a cool room (4°C), to prevent oxidation before it was decanted from the lees, with sodium metabisulphite solution (1 ml of 10%) added to every 1 litre of wine before bottling.

2.3 Grape sample preparation and analysis

The preparation of grapes, and subsequent extraction of residues by a system of passive diffusion through polymeric membranes, and analysis by a Varian model 3600 gas chromatograph with a dual thermionic detector and a DB-17 megabore column (30 m × 0·53 mm ID, 0·25 μm film thickness) (Varian, Mulgrave, Australia) was undertaken using a previously published method.[6]

2.4 Analysis of wine

A sample of wine (500 ml) taken within 14 days of wine-making was mixed with dichloromethane (100 ml) in a 1-litre separatory funnel and shaken (1 min) after which the layers were allowed to separate. The bottom solvent layer was transferred to an evaporator through a bed of anhydrous sodium sulfate (25 g). The extraction was repeated with fresh dichloromethane (50 ml) and the extracts combined. During evaporation hexane (5 ml) was poured into a Kuderna-Danish assembly through a Schnyder column, and repeated three times to ensure that all of the dichloromethane had been removed. After concentration and exchange of the solvent with hexane the volume was made up to 10 ml, sealed in a polyethylene membrane, and dialysed (24 h) against cyclohexane (80 ml). The cyclohexane and washes were then concentrated in the evaporator and the solvent exchanged with hexane during evaporation. The volume was made up to 5 ml and a sample (3 μl) analysed by GC.

2.5 In-vivo accumulation of azinphos-methyl by barley

Barley seeds were germinated in the laboratory on wet blotting paper in a tray and grown to a height of 10 cm. They were then transferred in batches (15–20 seedlings) to three beakers containing azinphos-methyl covering only the roots (10 ml of 1 + 1 by volume) at three concentrations (5, 10 and 15 μg ml^{-1}). Each test was replicated three times. After seven days the seedlings were removed from the insecticide mixtures and washed in running tap water to remove all the surface insecticide from the roots. Samples were then dried using a paper towel, weighed and extracted with dichloromethane and ethyl acetate (1 + 1 by volume) in a blender. After concentration and clean-up as described above the extracts were analysed for azinphos-methyl by GC. Data were analysed using 'Slidewrite 3' statistical software.

3 RESULTS AND DISCUSSION

The method of analysis was validated by spiking crushed grapes with azinphos-methyl at the rate of 0·3, 0·5, 1·0 and 2·0 mg kg^{-1} with each level replicated six times. The mean recovery of azinphos-methyl from grapes was 110·43% (range 96·5–124·2%) with a coefficient of variation of 9·53 (range 3·05–15·09).[6] The surrogate recoveries from the treated field samples ranged from 74·5 to 103·5%. The recoveries of azinphos-methyl from wine spiked at 1 and 2 μg litre^{-1} and triphenylphosphate (surrogate 0·32 μg litre^{-1}) were 93·9 and 96·4% respectively. These recoveries, along with the coefficient of variation data, show that this method of azinphos-methyl analysis for both grapes and wine will produce reliable results.

Half and twice the recommended concentrations of both formulations of azinphos-methyl were included to study the relationship between treatment concentration and residue levels in both grapes and in wine. The data show that there is a strong correlation between treatment concentration of azinphos-methyl and residue level in grapes (Tables 1 and 2) and in wine made from the treated grapes (Table 3). The relationship in grapes can be expressed as a decay function by exponential equation (Table 4). The correlation of values were lower in 1993/94 (range 0·6630–0·9279), when treatments were applied by commercial spraying equipment than in 1994/95 (range 0·9063–0·9722), when applied by hand-held sprayer. Residue levels in grapes resulting from all treatments applied by commercial sprayer in 1993/94 were below 2·0 mg kg^{-1} (MRL in Australia), including the first week after the final application (Table 1). In contrast, in 1994/95 when treatments were hand-applied, residues in grapes greatly exceeded this level. Excessive residues were recorded for the 1·2 and 2·4 g litre^{-1} WP treatments and all of the SC treatments, with residues from these treatment concentrations still in excess of the Australian MRL five weeks after the final application (Table 2). In a comparison of application methods, where treatments were hand-applied in 1994/95, in most cases residues on grapes were recorded in excess of 10-fold greater than those recorded in 1993/94, when treatments were applied by high-volume sprayer. Fluctuations in residues recorded between consecutive weeks in both years were not significantly different.

Azinphos-methyl is described as non-systemic;[1,2] however, on the basis of these results, it is suggested here that absorption of chemical by the leaves and translocation through the grapevine may have occurred. Fluctuations in the residue level in grapes in both formulations in consecutive weeks in the 1993/94 data would indicate that this may have occurred. A study into the uptake of azinphos-methyl by barley seedlings grown in solutions of various concentrations was undertaken to test this hypothesis. A strong correlation between the residues accumulated by barley and the

TABLE 1
Azinphos-Methyl Residue in Grapes after Treatment with Wettable Powder and Suspension Concentrate Formulations by High-Volume Sprayer, 1993/94

		Azinphos-methyl residue (*mg kg*$^{-1}$) ($\pm$*SE*)				
Treatment	*n*[a]	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*
Wettable powder (g formulation litre^{-1})						
0·7	3	0·376 ($\pm$ 0·06)	0·830 ($\pm$0·50)	0·259 ($\pm$0·02)	0·117 ($\pm$0·10)	0·081 ($\pm$0·06)
1·2	3	0·712 ($\pm$0·21)	0·633 ($\pm$0·37)	0·721 ($\pm$0·45)	0·226 ($\pm$0·07)	0·201 ($\pm$0·07)
2·4	3	1·154 ($\pm$0·05)	0·802 ($\pm$0·32)	0·909 ($\pm$0·62)	0·437 ($\pm$0·25)	0·202 ($\pm$0·07)
Suspension concentrate (ml formulation litre^{-1})						
1·2	3	0·502 ($\pm$0·25)	0·507 ($\pm$0·23)	0·234 ($\pm$0·06)	0·115 ($\pm$0·04)	0·113 ($\pm$0·06)
2·4	3	0·713 ($\pm$0·11)	0·868 ($\pm$0·13)	0·767 ($\pm$0·38)	0·226 ($\pm$0·06)	0·277 ($\pm$0·02)
4·8	3	1·931 ($\pm$0·18)	1·079 ($\pm$0·36)	1·910 ($\pm$0·63)	0·985 ($\pm$0·11)	0·955 ($\pm$0·12)

[a] *n* = number of determinations per mean.

TABLE 2
Azinphos-Methyl Residue in Grapes after Treatment with Wettable Powder and Suspension Concentrate Formulations by Hand-Held Sprayer 1994/95

		Azinphos-methyl residue ($mg\ kg^{-1}$) ($\pm SE$)				
Treatment	*n*[a]	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*
Wettable powder (g formulation $litre^{-1}$)						
0·7	3	3·81 (±1·20)	2·21 (±0·90)	1·96 (±0·20)	1·61 (±0·50)	1·64 (±0·05)
1·2	3	9·67 (±2·50)	7·09 (±1·80)	4·15 (±3·90)	4·34 (±2·60)	2·05 (±0·70)
2·4	3	15·54 (±4·40)	10·52 (±0·70)	8·73 (±0·30)	3·31 (±1·60)	2·79 (±0·50)
Suspension concentrate (ml formulation $litre^{-1}$)						
1·2	3	4·26 (±0·7)	3·83 (±0·7)	3·04 (±0·4)	1·95 (±0·5)	2·04 (±0·3)
2·4	3	11·66 (±1·8)	8·14 (±1·7)	5·81 (±1·8)	2·41 (±0·3)	2·27 (±0·3)
4·8	3	15·94 (±6·5)	13·32 (±0·7)	10·26 (±0·8)	2·36 (±3·4)	2·98 (±0·3)

[a] n = number of determinations per mean.

TABLE 3
Azinphos-Methyl Residues in Wine Made from Grapes Treated with Wettable Powder and Suspension Concentrate (SC) Formulations by High-Volume Air-Blast Sprayer, Analysed in the Year of Wine Production 1993/94, and Again in the Following Year 1994/95

Treatment (WP) (*g formulation* $litre^{-1}$)	*Residue* (*mg* $litre^{-1}$)	*Treatment (SC)* (*ml formulation* $litre^{-1}$)	*Residue* (*mg* $litre^{-1}$)	*Treatment*	*Residue* (*mg* $litre^{-1}$)
1993/94					
0·7	0·032	1·2	0·019	MC	0·055
1·2	0·041	2·4	0·017	Control	<0·001
2·4	0·107	4·8	0·160	Surrogate[a]	95·15%
1994/95					
0·7	0·188	1·2	0·199	Control	<0.012
1·2	0·519	2·4	0·504	MC	NA[b]
2·4	1·754	4·8	1·588	Surragate[a]	95%

[a] Triphenylphosphate (0·32 mg kg^{-1}).
[b] NA = not analysed.

TABLE 4
Exponential Relationship for Decay Rates of Various Treatments of Two Formulations of Azinphos-Methyl Applied to Grapes in 1993/94 and 1994/95

	Wettable powder 1993/94		*Wettable powder 1994/95*	
Treatment (*g* $litre^{-1}$)	*Equation*	*Correlation coefficient*	*Equation*	*Correlation coefficient*
0·7	$Y = 1{\cdot}0745e^{-0{\cdot}5024}$	0·8568	$Y = 3{\cdot}879e^{-0{\cdot}2003}$	0·9180
1·2	$Y = 1{\cdot}2519e^{-0{\cdot}3559}$	0·8728	$Y = 14{\cdot}087e^{-0{\cdot}3593}$	0·9180
2·4	$Y = 2{\cdot}0294e^{-0{\cdot}4093}$	0·9221	$Y = 26{\cdot}433e^{-0{\cdot}4591}$	0·9687
	Suspension concentrate 1993/94		*Suspension concentrate 1994/95*	
Treatment (*ml* $litre^{-1}$)	*Equation*	*Correlation coefficient*	*Equation*	*Correlation coefficient*
1·2	$Y = 0{\cdot}8537e^{-0{\cdot}4140}$	0·9279	$Y = 5{\cdot}481e^{-0{\cdot}2148}$	0·9525
2·4	$Y = 1{\cdot}3070e^{-0{\cdot}3237}$	0·8112	$Y = 19{\cdot}094^{-0{\cdot}4490}$	0·9722
4·8	$Y = 2{\cdot}0142e^{-0{\cdot}1499}$	0·6630	$Y = 31{\cdot}586e^{-0{\cdot}5080}$	0·9063

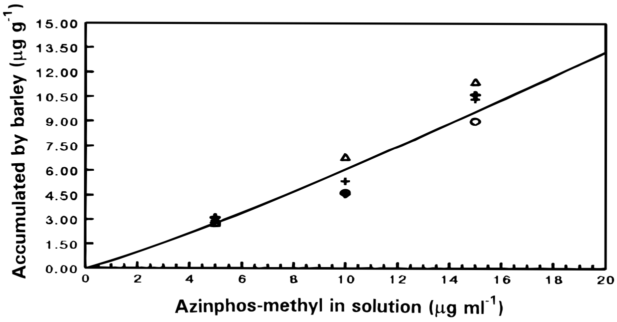

Fig. 1. Linear relationship between azinphos-methyl in aqueous solution and accumulated residue in barley seedlings. (+) Mean, (△) Replicate 1, (○) Replicate 2, (**+**) Replicate 3.

concentrations of azinphos-methyl in solution confirmed the suspicion of systemicity (Fig. 1). The relationship can be expressed by a linear regression ($r = 0{\cdot}985$) suggesting that azinphos-methyl was likely to have been translocated in the grapevine following foliar application. This may explain the fluctuating levels recorded after the final application, particularly in 1993/94, when treatments were applied by commercial sprayer, but also in 1994/95, when hand-applied treatments also came into contact with foliage.

Data on azinphos-methyl residues in wine made from treated grapes in 1993/94 and 1994/95 are given in Table 3. In 1993/94, mean residue levels ranged from 0·017 to 0·16 mg litre^{-1} depending on the treatment concentration. At the recommended rate in both formulations (1·2 g litre^{-1} WP and 2·4 ml litre^{-1} SC) residue levels in the wine were well below the Australian MRL developed from residues on grapes. Interestingly, while residues in wine were 5·9–29·6-fold higher in 1994/95 than were recorded in 1993/94, generally speaking levels were higher with the WP formulation in 1993/94, and with the SC formulation in 1994/95. The higher levels recorded in 1994/95 could be attributable to the deliberate targeting of grape bunches with hand-applied insecticide in that year, as opposed to the general vine spraying of the commercial sprayer in 1993/94. Wine made from grapes treated with azinphos-methyl by commercial sprayer in 1993/94, and re-analysed in 1994/95 after 12 months bottle storage, was found to have no detectable residues of azinphos-methyl. It is reasonable to conclude that, despite the combination of surface and translocated azinphos-methyl residues in the grape berries, if applied at the recommended rate with a commercial sprayer, the MRL for wine in Australia is unlikely to be exceeded. With regards to wine exports, of the nine countries currently importing Australian wine which have MRLs for azinphos-methyl, only Denmark and Sweden (0·5 mg kg^{-1}) had a level below that recorded for either azinphos-methyl formulation in this study. However, storage would seem to be an appropriate method of eliminating minor residues of this chemical in wine, thus overcoming any perceived difficulties due to detectable traces of insecticide.

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